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Short communication

β-Glucan plus ascorbic acid in neonatal calves modulates immune functions with and without *Salmonella enterica* serovar Dublin☆

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ABSTRACT

To determine if β-glucan plus ascorbic acid affects adherence and pathogenicity of *Salmonella* Dublin and innate immune response in neonatal calves, 20 calves were fed control or supplemented diets (β-glucan, 0.9 g/d, plus ascorbic acid, 500 mg/d) until d 23. On d 21, 5 calves per treatment received 2.4×10^8 CFU of *S.* Dublin orally. *S.* Dublin spread through intestinal tissues into mesenteric lymph nodes (MLN), spleen, and lung tissues within 48 h. All supplemented calves had less mRNA expression of IL-1 receptor antagonist in liver. Leukocyte cell surface markers changed in lung cells, but not in blood, MLN, or spleen. CD14 in lungs was greatest for calves receiving supplement and challenge, but CD18 in lungs was greater for challenged than control calves. Lung DEC205 was greatest for challenged calves with and without supplement compared to controls, but more lung cells expressed CD14 for all treated groups compared to controls. These data show that *S.* Dublin briefly inhabited the intestinal tract, moving quickly to spleen, MLN, and lung tissues. Lung tissue was modulated by *S.* Dublin, but supplement alone increased CD14 expressing cells. The supplement appears not to attenuate invasiness but modified some lung cell populations by 48 h.

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1. Introduction

Neonatal calves' intestinal tracts are developing immune protective mechanisms (Reber et al., 2008) as passive and innate active components of immunity provide protection (Firth et al., 2005). Colostrum has an abundance of bacterial contaminants (Fecteau et al., 2002), including gram negative rods, such as *Salmonella* which are frequently found in colostrums (Houser et al., 2008). *Salmonella* Dublin, a host-adapted serovar, is a problematic pathogen for young calves. Host specific pathogens do not behave as many of the enteric *Salmonella* serovars (Wallis et al., 1995; Mizuno et al., 2008). Little enteric disturbance is seen in calves after an infection with *S.* Dublin, but within 2 wk, respiratory or neurological complications can occur (Mizuno et al., 2007, 2008). Although vaccines have been tested, none to-date provided complete protection in calves (Mizuno et al., 2008), although one was 100% protective in mice (Mizuno et al., 2007). Early stimulation of innate immunity is a promising method to enable calves to thrive after maternal antibodies have waned and before their own adaptive responses are developed. In some instances, immunity of neonates can be stimulated by dietary supplements. One supplement that is promising is the combination of β-glucan plus ascorbic acid. Therefore, the objective of this study was to determine if a dietary supplement, β-glucan plus ascorbic acid, affects adherence or pathogenicity of *Salmonella* Dublin and innate immune responses in neonatal calves. The combination has been found to modulate innate immunity (Eicher et al., 2006).

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2. Materials and methods

2.1. Animals and experimental design

Animal care and use were approved by the Purdue Animal Care and Use Committee. Twenty calves were assigned to treatments in a 2 × 2 factorial arrangement of a *S.* Dublin challenge and a diet supplement consisting of yeast β-glucan extract plus ascorbic acid.

At 3 ± 1 d of age, calves were placed on control (CNT; no supplement) or supplement (BGA; β-glucan from Biothera, Inc. added at 0.9 g/d plus ascorbic acid, Stay-C from DSM Nutritional Products added at 500 mg/d) diets, continuing throughout the experiment (d 23). The β-glucan product was an extract from *Saccharomyces cerevisiae* that contains 70% β-glucans. On d 21, ten calves (5 control and 5 supplemented) received 2.4×10^8 CFU of *S.* Dublin orally in 1 ml of Luria–Bertani (LB) broth (Becton Dickinson and Company, Sparks, MD), followed immediately with 5 ml of sodium bicarbonate (4.0 g/100 ml, Sigma, St. Louis, MO) solution. After 48 h, calves were euthanized with Beuthanasia D (390 mg pentobarbital sodium and 50 mg phenytoin sodium/ml; Burns-Biotec Laboratories, Inc., Omaha, NE) i.v. injection. Daily rectal temperatures were recorded each morning.

2.2. Inoculum preparation

Salmonella enterica serovar Dublin originally isolated from a clinical bovine sample was provided by Dr. C. C. Wu from the Purdue University Animal Disease Diagnostic Laboratory, West Lafayette, IN. Stock cultures were grown in LB broth and maintained at −80 °C in 20% glycerol. The frozen stock (1 ml) was used to inoculate 9 ml of LB broth, and incubated at 37 °C overnight (statically). The overnight culture was used to inoculate (1%) fresh LB broth, and incubated at 37 °C on a rotating platform ($1.77 \times g$) for 4 h. The culture was harvested by centrifugation ($5000 \times g$), the supernatant was discarded, and the pellet was resuspended in phosphate-buffered saline (PBS; Sigma–Aldrich Co., St. Louis, MO) to achieve an optical density of 0.400 at 600 nm for an approximate final count of 2.4×10^8 CFU/ml. The actual final concentration was determined by plating onto XLT-4 agar (Neogen Corporation, Lansing, MI).

2.3. Microbial sample collection and processing

Individual rectal swabs were collected on the day of arrival, one week later, and immediately prior to the bacterial challenge to monitor for pre-existing *Salmonella* infection. No pre-existing *Salmonella* infection was found. Samples collected during the necropsies included; rectal swab, ileal loops (to obtain ileal contents, mucosal scrapings, and ileal tissue), mesenteric lymph nodes (MLN), cecal contents, spleen, and lung.

All samples were processed for isolation and identification of *S. enterica*, including sequential enrichment in Tetrathionate broth (Neogen Corporation, Lansing, MI) at 37 °C for 24 h, and Rappaport–Vassiliadis broth (Neogen Corporation, Lansing, MI) containing 50 μg/ml of novobiocin (Sigma–Aldrich Co., St. Louis, MO) at 42 °C for 24 h, and isolation on XLT-4 agar plates (37 °C for 24 h). Presumptive *Salmonella* colonies on XLT-4 agar were individually picked and transferred onto Rambach chromogenic agar (DRG International, Mountainside, NJ), and incubated at 37 °C for 24 h for identification. Rectal swabs, ileal contents (1 g), and cecal contents (1 g) were directly placed in tubes containing 10 ml of Tetrathionate broth, and cultured as described above. All tissue samples (ileal tissue, MLN, spleen, and lung) were immersed in alcohol and flamed for external decontamination, smashed and homogenized with a Stomacher (Seward, Worthing, UK), and cultured as described above.

2.4. Ileal loop assay for attachment of Salmonella enterica serovar Typhimurium

S. enterica serovar Typhimurium were grown statically overnight in LB broth. Bacterial cells were harvested by centrifugation at $6000 \times g$ at 4 °C for 15 min, and were washed 3 times in equal volumes of sterile PBS. Cells were resuspended in Dulbecco's Modified Eagle Medium + l-glutamine (DMEM, Mediatech, Herndon, VA) to an optical density (600 nm) of 0.4 (approximately 1×10^6 cells/ml as determined by a prior standard curve). The inoculum was serially diluted and plated in LB broth to obtain the actual number of cells in the inoculum.

A 10-cm section of the ileum was taken, starting 3 cm proximal to the ileo–cecal junction, from each calf for an ex vivo ileal challenge assay and was gently flushed with 0.05 M PBS (pH 7.2). Tissue sections were immediately placed in ice-cold DMEM and kept on ice until used for the in vitro *Salmonella* challenge assay. The organ culture procedure of Burkholder et al. (2008) was used. Briefly, ileal sections were removed from DMEM, sealed at one end with 35-mm dialysis clamps, and inoculated with approximately 6 ml of *S.* Typhimurium culture suspended in DMEM. The open end of the ileal section was sealed with dialysis clamps, the exterior was rinsed with PBS, and the ileal loops were incubated in 100 ml of DMEM for 1 h at 37 °C in a rotary water bath in a 10% CO_2 atmosphere. After incubation, ileal contents were removed, the interior and exterior of each section was rinsed with PBS, tissues were homogenized (T-25 basic homogenizer, Ultra-Turrax, IKA-Werke), serially diluted in buffered peptone broth, and plated on XLT4 agar plates. Plates were incubated at 37 °C for 24 h and were enumerated for *Salmonella*; counts (log 10 CFU) of *Salmonella*/g of tissue were calculated.

2.5. Tissue collection for qRT-PCR

A 2 cm cubed lung section was excised from the dorsal portion of the caudal lobe. A cross section of the spleen at mid-spleen (1 cm wide) and MLN near the ileo–cecal junction were collected. Beginning at the ileo–cecal junction, a proximal 4 m section along the length of the small intestine was marked; this indicated a representative and repeatable site for jejunal extraction. A 2 cm section of jejunal tissue was excised and cut along the mesenteric side. The ileum was cut 10 cm proximal to the ileo–cecal junction. Samples were collected as for the jejunum. From the apex of the cecum, a 2 × 2 cm section of tissue was collected. Intestinal

tissues were rinsed with sterile 1× Hank's Balanced Salt Solution (HBSS; Invitrogen Corporation, Carlsbad, CA) and all tissues were placed immediately into RNALater.

2.6. qRT-PCR

Extraction was performed on tissues that were preserved in RNALater using QIAmp RNA Mini Kit (Qiagen, Valencia, CA). Reverse transcription was performed on 100 μg per ml of RNA using TaqMan® Reverse Transcription Reagents (Applied Biosystems, Foster City, CA) and cDNA synthesized using the following program: (1) 50 °C for 2 min; (2) 95 °C for 10 min; (3) 95 °C for 15 s; (4) 60 °C for 1 min; (5) steps 3 and 4 were repeated for 30 cycles; (6) the final stage was 60 °C for 5 min with a holding temperature of 4 °C. On completion of reverse transcription, samples were stored at −80 °C until amplification.

Primer and probe sequences for quantitative real time-PCR (qRT-PCR) were designed using Primer express 1.1 Software (Applied Biosystems) and synthesized by Applied Biosystems (Foster City, CA, US; Table 1). Probes were labeled with VIC fluorescent dye. Five 5 μl of template cDNA was used for RT-PCR for each gene. Each sample was combined with 4.5 μl (10 μM) of forward and reverse primer, 3.25 μl probe (3.85 μM), 7.75 μl DEPC water, and 25 μl Master Mix (TaqMan® Universal PCR Master Mix, Applied Biosystems, Foster City, CA) for the control genes. All qRT-PCR reactions were performed in duplicate using template from individual animals in each reaction. The relative standard curve was constructed (in triplicate) for quantification using dilutions of cDNA (1, 0.5, 0.25, 0.125, 0.0625, and 0.0315). Quantitative RT-PCR was performed and analyzed using ABI prism 7000 sequence detection system (Applied Biosciences, Foster City, CA) with the following program: (1) an initial step at 50 °C for 2 min; (2) followed by 10 min step at 95 °C; (3) then 20 s at 95 °C; (4) 1 min at 60 °C; (5) repeat steps 3 and 4 for 50 cycles. Commercially available eukaryotic 18S rRNA (Applied Biosystems, Foster City, CA) primers and probe were used as endogenous control.

Table 1
Sequences for primer and probes for qRT-PCR analysis of lung, liver, spleen, and mesenteric lymph node tissues.

Gene	Primer or probe	Sequence 5′–3′
IL-1	Forward	TTCCTGTGGCCTTGGGTATC
	Reverse	TGGGCGTATCACCTTTTTTCA
	Probe	CAAGAATCTATACCTGTCTTGT (VIC/MGB)
IL-1Ra[a]	Forward	CCTCCTTTCTCACCCCAGATC
	Reverse	AGAAAATGGAAGCCGCTTAGG
	Probe	CAGGCGCTCACTTC (VIC/MGB)
TLR[b]4	Forward	CCGGATCCTAGACTGCAGCTT
	Reverse	TCCTTGGCAAATTCTGTAGTTCTTG
	Probe	CCGTATCATGGCCTCT (VIC/MGB)
IL-12	Forward	CAGCAAGCCCAGGAAGGA
	Reverse	TGACAGCCCTCAGCAGGTTT
	Probe	TGCCTCGACTACTCC (VIC/MGB)
TLR2	Forward	CCACGGAAGGAGCCTCTGA
	Reverse	GCCATCGCAGACACCAGTT
	Probe	CAGGCTTCTTCTCTGTCTT (VIC/MGB)
IFN-γ	Forward	ACCTCCTTGGGACCTGATCAT
	Reverse	GAGAGAGTTAGGCCGGAGTAGTTG
	Probe	CACAGGAGCTACCGATT(VIC/MGB)

[a] Ra = receptor antagonist.
[b] TLR = toll-like receptor.

2.7. Leukocyte phenotyping

Five hundred microliters of whole blood were aliquoted into four 5-ml polypropylene tubes: unstained control, CD18-FITC (20 μl/sample per manufacture's instructions, BDPharmingen, San Jose, CA); CD14-FITC (20 μl of 100 mg/ml; DAKO, Carpenteria, CA); and DEC205-FITC (0.002 mg/sample in 20 μl; AbD Serotec, Raleigh, NC). Samples were incubated for 30 min at 37 °C, RBC were lysed by hypotonic lysis, washed once in HBSS and resuspended in 500 μl of HBSS. Coulter EPICS XL-MCL Flow Cytometer and System II software (Beckman Coulter Co., Miami Lakes, FL) were used to analyze 10,000 cells from each sample with a 488 nm air cooled argon laser for excitation, a 525 band pass filter for detection of FITC emissions.

2.8. Statistical analysis

Treatment differences of ileal loop assay were determined and analyzed using the GLM procedure in SAS (SAS Inst. Inc., Cary, NC) testing for treatment and tissue differences. *Salmonella* prevelence data were analyzed by Chi-square analysis. All other data were analyzed using the mixed models procedures of SAS (SAS, V9.1.3, SAS Institute

Table 2
Supplement of a *Saccharomyces cerevisiae* extract (70% β-glucan; 0.9 g/d) plus ascorbic acid (500 mg/d) effect on number of samples positive for of *Salmonella* 48 h after an oral challenged with *Salmonella* Dublin and LS means of CFU of *Salmonella*/g of tissue from jejunal and ileal loops incubated in vitro with *Salmonella enteriditus* serovar Typhimurium for 24 h.

Treatments	Rectal swab	Ileal contents	Ileal mucosa	Ileal tissue	MLN	Cecal contents	Spleen	Lung
Control	5/5[a]	2/5	3/5	4/5	5/5	2/5	5/5	5/5
Supplement	2/5	0/5	4/5	4/5	4/5	1/5	5/5	4/5
Total	7/10	2/10	7/10	8/10	9/10	3/10	10/10	9/10

Treatments	*S.* Typhimurium, CFU	Location	*S.* Typhimurium (CFU)
Control	7.85	Jejunum	7.48
Supplement	7.46	Ileum	7.84
SE	0.201	SE	0.285
Probability of treatment differences	0.210	Probability of location differences	0.234

[a] Number of positive samples over total number of samples.

Inc., 1989). Data were analyzed as a randomized complete block design with repeated measures. Fixed effects of the model were treatment, sampling point (day), and interactions of treatment with day used as a repeated measure. Calf was considered the experimental unit. Table 2 shows log 10 CFU/ml transformed data. Other data presented in this paper show the non-transformed values of the data, however p-values were calculated using log 10 transformed data for all liver and spleen genes, for all MLN genes except Il-1, TLR2 and TLR4, and for all lung genes except IL-12, IFN-γ, TLR2, and TLR4. Statistical differences were reported when p values were <0.05 and tendencies towards significance when ($0.05 < p < 0.1$). Data are presented as Least Squares Means ± SE.

3. Results and discussion

3.1. General health

Salmonella Dublin infection was attained with a field isolate and verified by change in calf rectal temperatures. At the end of the second week, 5 calves (2 from the supplemented group and 3 from the control diet group) required a broad spectrum antibiotic, which was cleared prior to the challenge. Rectal temperatures were different ($p < 0.05$) between challenged and CNT by 24 h, and differences were more pronounced by 48 h ($p < 0.01$). Similar to the current study, others have shown peak temperatures occurred at 48–72 h after inoculation (Watson et al., 2000). There were no differences in rectal temperatures between supplemented or not supplemented calves ($p > 0.10$).

3.2. Microbial analysis

Salmonella presence corresponded to the *S.* Dublin challenge. All unchallenged tissues were negative for *Salmonella* throughout the study. However, no differences ($p \geq 0.10$) were evident between supplemented and control calves for *Salmonella* in any of the samples (Table 2). In challenged calves, greatest counts frequency was found in the spleen (10 of 10); followed by lung and mesenteric lymph nodes (9 of 10 in each tissue). Few samples contained *Salmonella* in the ileal contents (2 of 10) or in cecal contents (3 of 10). Rectal swabs and ileal mucosa each had 7 of 10 calves positive for *Salmonella*, and ileal tissue was positive in 8 of 10 calves.

Salmonella Dublin is a host-specific pathogen that can be particularly invasive in cattle. Invasiveness has been associated with several gene mutations that may be responsible for species specificity (Watson et al., 1995; Pullinger et al., 2008). Here, we have shown that within 48 h, *S.* Dublin located to spleen, lung, and MLN tissues. Additionally, invasiveness is more severe with stress hormones, cortisol, or the chatecholamines such as norepinephrine (McCuddin et al., 2008). These observations may explain why outbreaks typically occur in calves during periods of greater stress, such as weaning. Therefore, our challenge at 3 week-

Table 3
Relative abundance (18S internal standard) of toll-like receptors (TLR) and cytokines determined by quatitative RT-PCR analysis of mesenteric lymph node (MLN), lung, spleen, and liver tissues. Data are LS means.

Tissue gene	Control	Supplement[a]	*S.* Dublin[b]	Supplement + *S.* Dublin	SE	Probability of trends and significant main effects and interactions
Liver						
IL-1	0.42	0.28	0.39	0.23	0.14	
IL-1 Ra[c]	0.31	0.14	0.52	0.13	0.13	Supp = 0.051
TLR2	0.58	0.45	0.54	0.45	0.18	
TLR4	0.83	0.41	0.45	0.46	0.30	
IL-12	0.21	0.06	0.05	0.04	0.08	
IFN-γ	0.67	0.32	0.18	0.16	0.25	
Lung						
IL-1	0.25	0.43	0.30	0.20	0.13	
IL-1 Ra	0.21	0.26	0.26	0.14	0.09	
TLR2	0.30	0.43	0.34	0.35	0.08	
TLR4	0.40	0.70	0.28	0.43	0.13	Supp = 0.056
IL-12	0.04	0.11	0.07	0.12	0.03	Supp = 0.077
IFN-γ	0.48	0.45	0.16	0.43	0.16	
Spleen						
IL-1	0.22	0.30	0.41	0.37	0.10	
IL-1 Ra	0.14	0.15	0.29	0.16	0.08	
TLR2	0.49	0.55	0.78	0.46	0.21	
TLR4	0.33	0.61	0.90	0.54	0.26	
IL-12	0.06	0.13	0.23	0.12	0.08	
IFN-γ	0.26	0.58	0.98	0.57	0.32	
MLN						
IL-1	0.38	0.32	0.30	0.44	0.12	
IL-1 Ra	0.17	0.18	0.22	0.39	0.13	
TLR2	0.52	0.50	0.53	0.46	0.06	
TLR4	0.48	0.57	0.62	0.42	0.14	
IL-12	0.15	0.09	0.14	0.10	0.21	
IFN-γ	0.40	0.49	0.77	0.53	0.30	

[a] *Saccharomyces cerevisiae* extract (70% β-glucan; 0.9 g/d) plus ascorbic acid (500 mg/d).
[b] Oral 2.4×10^8 *S.* Dublin.
[c] Ra = receptor antagonist.

of-age was appropriate for neonatal calves as maternal antibodies are waning.

We also tested *S.* Typhimurium (non host specific) ability to attach, to contrast with the host specific *S.* Dublin. Control calves did not differ ($p = 0.21$) from BGA calves for CFU of *S.* Typhimurium after a 24-h challenge in ileal loops (Table 2) from uninfected calves. Differences in *S.* Typhimurium CFU were not detected ($p = 0.23$) between jejunal and ileal loops.

Others (Watson et al., 1995) have not found differences between *S. typimurium* or *S. dublin* in ileal loop assays.

3.3. qRT-PCR

Treatments did not affect expression of cytokines and TLR2 and TLR4 in spleen and mesenteric lymph node tissues (Table 3). However, supplemented calves has less (supplement main effect, $p = 0.05$) Il-1 receptor antagonist mRNA expression in liver tissues and tended to have greater (supplement main effect, $p = 0.056$) TLR4 and Il-12 (supplement main effect, $p = 0.077$) mRNA expression in lung tissues. Toll-like receptors agonist, particularly CpG, increased phagocytic capacity towards *Salmonella* Typhimurium (Wong et al., 2009). Thus we hypothesized that a combination of β-glucan from *S. cerevisiae* and ascorbic acid that have immunomodulating properties would enhance enteric or systemic mucosal responses. TLR4 and IL-12 both tended to be up-regulated in lung tissues by supplementation, suggesting that supplement had modified immune responses in the lung. However, the response was not altered by the *S.* Dublin challenge.

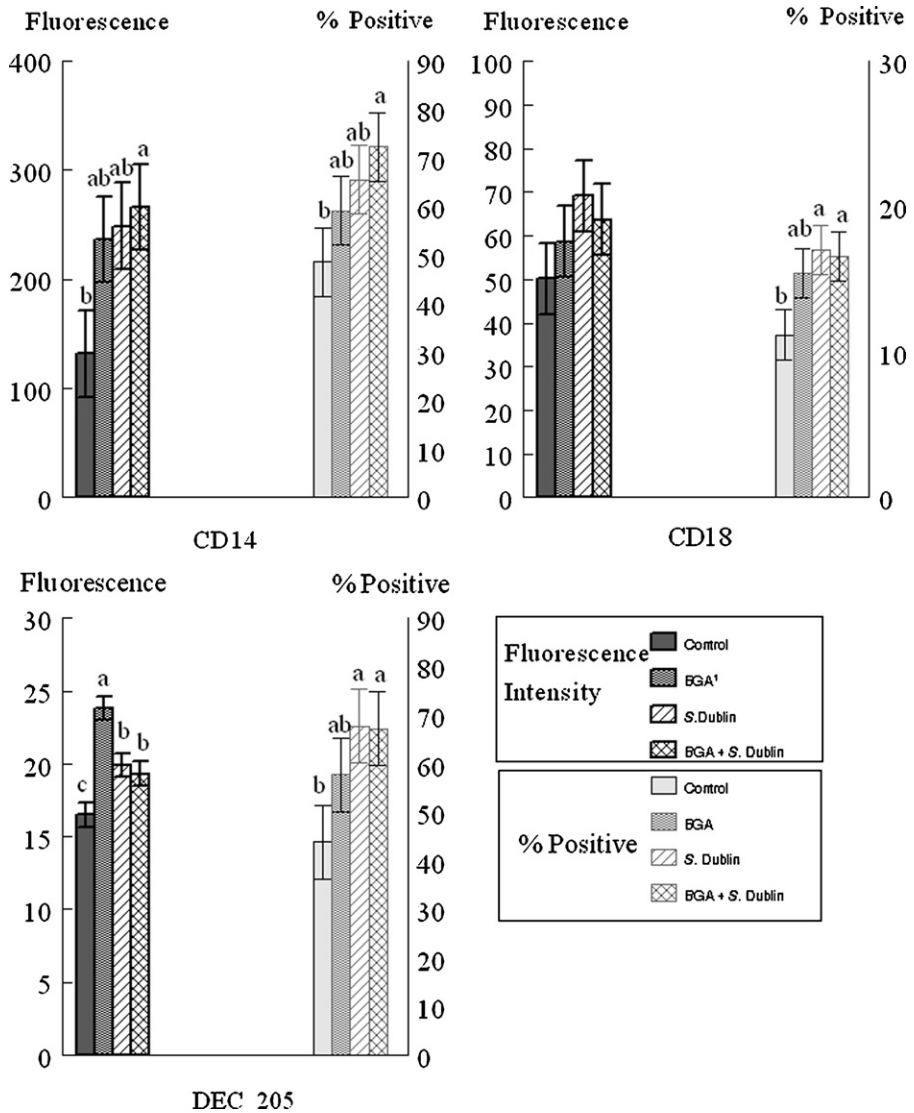

Fig. 1. Percentage of lung cells positive (right axis, right bars) and fluorescence intensity of positive cells (left axis, left bars) for cell surface expression of CD14, CD18, and DEC205 of calves fed a supplement (BGA[1]) of *Saccharomyces cerevisiae* extract (70% β-glucan; 0.9 g/d) plus ascorbic acid (500 mg/d) or non-supplemented control calves (Control) and challenged at 21 days-of-age with a field strain of *S.* Dublin (*S.* Dublin) or control inoculum. Data are LS means ± SE. [a,b,c]Means without common superscript differ ($p \leq 0.05$).

In swine, mononuclear phagocytic cells only differed in mRNA expression of TLR2, but not TLR4 or TLR9 and expression did not differ for the host-specific *S.* Choleraesuis and *S.* Typhimurium (Burkey et al., 2009). Up-regulation of TLR4 suggests that the supplement may provide some support for an earlier immune response to infection by respiratory pathogens. β-Glucan treatment in rats (Bedirli et al., 2007) provided a protective effect for lung tissues after cecal ligation and puncture purportedly by reduced cytokine expression. However, responses to β-glucan can be quite varied, depending on the extraction process, final size, and solubility (Sonck et al., 2010). Whether the pathogens that were found in lung tissues in this study were hidden from immune recognition or whether virulence and recognition molecules have been altered in the process of getting to the lungs is not clear. No evidence of immune responses to the *S.* Dublin was found in any of the tissues examined here within 48 h of the challenge. However, changes in cell populations within the lung tissues were evident by 48 h post inoculation and pyrexia was evident between 24 and 48 h.

3.4. Leukocyte phenotyping

Percentage of cells positive for the adhesion molecule, CD18 and fluorescence intensity of positive cells were not different for total leukocytes, MLN, or spleen cells (data not shown). However, lung tissue of *S.* Dublin challenged calves had a greater percentage of cells fluorescing (*S.* Dublin main effect; $p = 0.05$) with CD18 antibody (Fig. 1), although fluorescence intensity of positive cells was not different among the treatments ($p > 0.10$).

Percentage of cells positive for expression of the CD14 portion of the LPS recognition molecule was only different in lung cells (Fig. 1). Calves treated with both supplement and *S.* Dublin challenge had the greatest ($p = 0.03$) number of cells expressing CD14 compared to controls, but were not different from the other 2 treatments. A trend for a similar pattern was observed in fluorescence intensity of positive cells ($p = 0.08$). No differences were detected among the treatments in leukocytes, MLN, or spleen tissues for CD14 expression.

Similarly, no treatment differences were detected in leukocyte, MLN, or spleen cells for the percentage of cells that were positive for the dendritic cell marker DEC205 or fluorescence intensity of positive cells. However, lung tissues of calves that receive the *S.* Dublin challenge had more ($p = 0.05$) cells positive for DEC205 fluorescence (Fig. 1). Curiously, calves that were treated with only supplement, had greater fluorescence intensity of DEC205 positive cells ($p < 0.0001$) than control calves and than the two *S.* Dublin treatments ($p < 0.005$). *S.* Dublin challenged calves also had greater DEC205 fluorescence intensity ($p = 0.01$) than control calves. Expression of CD14 showed that some cell population changes were occurring in the lung, but not in other tissues that we examined. When both the challenge and supplement were given, both percentage of cells and fluorescence intensity for CD14 increased. In contrast, only the percentage of cell expressing CD18 was increased by *S.* Dublin, suggesting an influx of activated leukocytes. It also appears that dendritic cell maturation was underway. Only *S.* Dublin caused more cells expression DEC205 to appear in lung tissues. Although those cells, also expressed more of the DEC205 than the control cells, supplement only (no *Salmonella*) had the greatest (DEC205) fluorescence which was attenuated with *Salmonella* challenge. This shows that the supplement was also modulating the maturation of dendritic cells in the lung regardless of the challenge.

In conclusion, we have shown rapid movement of *S.* Dublin from the intestinal tract to the lungs, spleen, and MLN by 48 h post-infection, but supplementation did not affect attachment or trafficking of *Salmonella.* Supplementation with β-glucan plus ascorbic acid modulated TLR4, cytokines, and cell populations in the lung and their activation. Additionally, it improved fecal scores of *Salmonella* challenged calves, but did not alter rectal temperatures. Mild activation could result in an earlier immune response; however that hypothesis still should be tested with a longer study.

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